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ADAPTATIONS TO ENVIRONMENTAL OXYGEN LEVELS IN INFAUNAL AND EPIFAUNAL SEA ANEMONES

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Numerous investigators have shown correlations between various physiological properties of aquatic organisms and the characteristic levels of oxygen in which the animals are found. Older studies on survival time under low oxygen conditions generally indicate that, within closely related groups, burrowing species are more resistant to oxygen deprivation than epifaunal forms (Packard, 1905). Similarly, animals living in fast-moving streams are less resistant than those living in relatively unmixed pond water (Fox, Simmonds and Washbourn, 1935; Bovbjerg, 1952; Walshe, 1948). Walshe (1948) also showed that, among the chironomid larvae, resistance to oxygen lack is better correlated with ecological distribution than with phylogeny.

Numerous studies support the very plausible notion that animals from low oxygen environments have a lower rate of oxygen consumption than their counterparts from high oxygen environments when compared at the same oxygen concentrations. Examples are two species of *Balanus* (Prasada Rao and Ganapati, 1968); epifaunal and infaunal tropical echinoids (Lewis, 1968); oxygen minimum layer mysids (Childress, 1971); stream and pond insect larvae (Fox, Simmonds and Washbourn, 1935), crustaceans (Fox and Simmonds, 1933) and leeches (Mann, 1956); and maldanid polychaetes (Mangum, 1963, 1964a).

Perhaps the most elusive physiological correlate of environmental oxygen level is the degree to which oxygen consumption rate is maintained constant over a range of ambient oxygen concentrations. It is clear that in many aquatic invertebrates respiratory regulation within a species is not entirely constant. It varies with temperature (Thomas, 1954; Wiens and Armitage, 1961), weight (Helff, 1928), molt cycle (Thompson and Pritchard, 1969) and previous activity levels (Nimura and Inoue, 1969).

Relatively few investigators have examined the metabolic response of aquatic animals to wide ranges of oxygen concentration following periods of oxygen deprivation. Instead, most investigations have characterized the response to anoxia only at oxygen concentrations at or near air saturation. Prosser, Barr, Pinc and Lauer (1957) have shown that goldfish respond to chronic exposure to low oxygen conditions by a reduction of standard metabolism and a shift of critical pO_2 to lower oxygen partial pressures, accompanied by increased hemoglobin concentrations and red blood cell counts. After oxygen lack, oxygen consumption rates in the mud snail *Nassarius obsoletus* increase (Kushins and Mangum, 1971), but the response

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of oxygen sensitivity is variable. While oxygen sensitivity may either decrease or show no change, the most common response is to increase. As yet, no clear picture has emerged of the response of marine invertebrates to wide ranges of oxygen concentration following chronic lack of oxygen.

Many comparative studies of adaptation to environmental oxygen level have been concerned only with selected aspects of the animals' responses to oxygen concentration. The present investigation is based on the premise that an understanding of the adaptations of animals to the prevailing levels of oxygen in their respective habitats requires consideration of oxygen distribution in the environment and some knowledge of the manner in which oxygen enters the animal, as well as the kinetics of its consumption in relation to stress situations. Thus, the present study has centered around various morphological, behavioral, and physiological aspects of adaptation to low oxygen in two species of sea anemones that are distinct ecologically with reference to the oxygen characteristics of their respective environments.

Methods and Materials

Habitat

Metridium senile (L.) is a characteristically epifaunal species attached to rocks and pilings in semi-protected areas of the New England coast and south to New Jersey. *Haloclava producta* (Stimpson) is a burrowing form living in medium grain sand; those animals used in this study were collected from Waquoit Bay, Massachusetts. Both species were provided by the Supply Department, Marine Biological Laboratory.

A sediment sample of 570 g (dry weight) containing 3 *H. producta* was taken in July 1971 at Waquoit Bay. The sample was rinsed, soaked in 1 N NaOH, rinsed again, and dried at 60° C for 24 hours. The dried sample was then sieved through a standard series (Mangum, 1964b) and each fraction weighed.

In the laboratory the burrow shape and orientation of *H. producta* was observed in a sandwich of sand held between two glass plates. Two to four animals were placed in the apparatus at the same time. They were allowed to burrow and were subsequently observed over a period of several weeks.

Behavior

Two facets of behavior in *Haloclava producta* were examined: (1) spontaneous activity of the column of intact animals and (2) irrigation of artificial burrows. Spontaneous activity of the column of intact animals was recorded by allowing each animal to adhere to the inside wall of a small piece of glass tubing and then hooking the free tentacular crown to a kymograph lever by the method of Batham and Pantin (1950a).

Irrigation activity of *H. producta* in straight glass tubes was kymographically recorded in the apparatus described previously (Mangum, 1964a). In addition a geometrical estimate of irrigation rate was made by calculating the volume of water pinched off in each peristaltic irrigation wave of an animal in a glass tube and multiplying this value by the frequency of the waves.

Distribution of oxygen

Field levels of oxygen concentration were determined in Waquoit Bay. Interstitial water samples of 25 ml were taken from depths of 5 cm and 10 cm in the sediment. The samples were drawn anaerobically into a 20 ml syringe fitted with a #13 gauge needle to prevent clogging with sand. A maximum of 0.5 ml sand was included in the sample. Immediately after the sample was taken, the plunger of the syringe was replaced with a calibrated galvanic oxygen probe (Precision Scientific Co.), leaving no air space, and the water was mixed by inverting the syringe and probe several times while holding the tip closed. The movement of introduced sand grains provided mixing and obliterated oxygen gradients around the probe head. Two samples were taken at each depth and several additional measurements were made on the oxygen content of the water overlying the sand. The results were similar to *in situ* measurements obtained by rotating the probe in the water or sand, although we were less confident in this case of adequate mixing.

Measurements of oxygen concentration in the running seawater system in the laboratory were made with a polarographic electrode (Yellow Springs Instrument Co. model 5420) and with the pO_2 module of a Radiometer Blood Gas apparatus (BMS1 equipped with acid-base analyzer PHM1). The oxygen content of glass tubes occupied by *Haloclava producta* was determined by removing 50 μl of water from each tube ($N = 15$) and measuring its pO_2 with the Radiometer Blood Gas apparatus.

The oxygen level in the gastrovascular fluid of *Metridium senile* equilibrated to running seawater was determined with the Radiometer apparatus on samples anaerobically removed from each of 10 large (diameter *ca.* 8–12 cm) animals with a 1 ml syringe. The oxygen content of gastrovascular fluid from *H. producta* burrowed in a few cm of sand and held in running seawater was similarly determined, but pooled samples from groups of three of these very small (*ca.* 0.5 cm) animals were used to make each of 2 determinations.

Survival

Survival of animals of the two species under low oxygen conditions was determined by placing them in darkened chambers containing seawater flushed with nitrogen. The initial concentration of oxygen in the chamber was 0.5–0.7 ppm. After introduction of the animals the chamber was sealed with a rubber or ground glass airtight stopper and kept at room temperature (22–24° C) for the desired dosage time. At the end of this period the animals were returned to running seawater until criteria for either mortality (decay and disintegration) or survival (muscular response to tactile stimulation) were met. Animals that survived were maintained in running seawater for an additional 24–48 hours before being definitively scored. Simultaneous controls were maintained in running seawater at 18–22° C.

Anatomy

Anatomical observations were made on the distribution of ciliated epithelial surfaces, complexity and bulk of internal structures, body wall thicknesses and

the distribution of external surface area in both species. Ciliary activity of the column and tentacles of living animals, and of the actinopharnyx and mesenterial filaments of freshly dissected animals, was observed with carmine and india ink suspensions. The arrangement and number of mesenteries and body wall thicknesses were determined on preserved and sectioned material. Animals of comparable column diameter were anesthetized with $MgSO_4$, fixed in Bouin's solution, embedded in paraffin, sectioned at the level of the actinopharynx, and stained with hematoxylin and eosin. In calculating external surface area the body wall and each individual tentacle were approximated by cylinders.

Metabolic response to diminishing concentrations of oxygen

In view of the findings of numerous investigators (Helff, 1928; Thomas, 1954; Van Dam, 1954; and Wiens and Armitage, 1961) that the magnitude of change of oxygen consumption rate with reduced oxygen concentrations is influenced by environmental factors, the metabolic response curve of *Metridium senile* was determined under a variety of conditions. Ten different conditions were produced by creating nine combinations of acclimation temperature and experimental temperature, and by exposing one group of animals to anoxia for 24 hours before determining metabolic response to diminished oxygen. These conditions and the number of animals in each group are given in Table I.

Individual animals were allowed to set in darkened respiration chambers of 30, 60, 120 or 300 ml volume. After a period of temperature acclimation (at least 3 days) the chambers were placed in a constant temperature water bath and were sealed by the insertion of a Yellow Springs Instrument Co. Model 5420 oxygen probe. The animals were allowed to reduce the oxygen concentration to a low level (at least below 33% air saturation), and the oxygen concentration monitored by a Model 54 meter (YSI) connected to a Model 80 chart recorder (YSI).

Weight specific oxygen consumption rates were calculated for each 15 min interval and analyzed as a function of the median oxygen concentration in the chamber during that interval. Linear and semilogarithmic regression coefficients were calculated for the relationship between oxygen consumption and oxygen concentration in each experiment. The regression coefficient b is an index of the magnitude of the change in oxygen consumption rate as a function of change in oxygen concentration, assuming a time-independent relationship. The use of the coefficient b as a quantitative description of the relationship between oxygen consumption rate and oxygen concentration is valid only if the slope of the response curve is not changed by the rate at which the animal encounters low oxygen conditions. If the slope of the response curve is affected by the rate of change in oxygen concentration, then the change in consumption rate divided by the change in concentration (this quotient being b) is inversely proportional to time.

To evaluate the possible importance of time dependence, each experiment was treated as a first order kinetic reaction and the corresponding rate constant (k) was calculated from the time required to reduce the oxygen concentration by 50% from the formula $k = 0.693/t_{\frac{1}{2}}$ where k has units of hr^{-1}. If time dependence is a factor in the metabolic response, b should be directly proportional to k. To describe the relationship, b was plotted as a function of k. The effect on b of the

various environmental conditions of the 10 experimental groups indicated in Table 1 is considered below.

Metabolic response to prolonged anoxia

Metridium senile and *Haloclava producta* were maintained in the laboratory for at least three days in running seawater at 17–19° C. They were allowed to set individually in darkened respiration chambers of 300 ml volume (*M. senile*) or 25 ml volume (*H. producta*). Each chamber was sealed with an oxygen probe and oxygen depletion was monitored until oxygen consumption reached zero at 17° C (± 0.05° C). The probe was then removed and the chamber resealed with either a rubber or ground glass stopper. After 24 hr the chamber was refilled with air saturated seawater and the depletion of oxygen monitored again until oxygen consumption reached zero.

For each pair of experiments (before and after exposure to 24 hr of anoxia) the consumption rate at each integral value of oxygen concentration (in ppm) was calculated from best fit semilogarithmic regression lines and both the absolute and per cent increases of the post-anoxic measurement over the initial measurement were determined. The statistical significance of these increases was evaluated using Student's *t* test for paired observations. The difference in oxygen concentration at which oxygen consumption reached zero was analyzed for each pair of experiments using the same test. In addition, the time required for each animal to reach a maximum rate of oxygen consumption following introduction into air saturated seawater after 24 hr exposure to anoxia was analyzed as a function of animal weight using regression analysis.

Results

Habitat

Waquoit Bay is a shallow, broad pan of water on the southern shore of Cape Cod, Massachusetts. The results of grain size analysis of *Haloclava producta* occupied sediment taken there indicate that the modal size class for the particles is 500–999 μ (42.61% dry weight) with nearly as large a fraction (41.91%) in the 250–499 μ range. These two size classes dominate the sediments at 5 of 6 other stations along the southern shore of Cape Cod (Mangum, Santos and Rhodes, 1968).

In the laboratory, *H. producta* burrows over a period of several hours to a depth of at least 10–15 cm. The animals attach the physal disc to the bottom of the observation apparatus and maintain the oral disc flush with the surface of the sand. In their burrows the animals are rather vermiform, extending over 10 cm in length and about 0.6 cm in diameter. The burrows, apparently not highly stabilized by secretory products, easily collapse.

If animals are left undisturbed in the glass plate apparatus for several weeks, organic detritus is deposited on the surface of the sand. A small area around each burrow, however, is kept clear of detritus by the feeding activities of the anemones. As in the case of *Phyllactis conchinata* (D. C. Mangum, 1970), non-burrowed *H. producta* do not respond to particulate food.

Behavior

The column of intact *Haloclava producta* shows a rhythmic pattern of contraction and relaxation similar to those already described for *Metridium senile* (Batham and Pantin, 1950a) and *Diadumene leucolena* (Sassaman and Mangum, 1970). The frequency of the pattern is 12 (± 1.3 S.E.) contractions/hr (3.5 hrs recording time).

H. producta also irrigates artificial burrows with cyclic peristaltic waves which originate below the tentacular ring and are propagated aborally. Observations of four different individuals revealed periodicities of 25, 25, 25 and 24 sec between pumps, with the waves traveling at approximately 0.2 cm/sec. Geometrical analysis of an anemone irrigating a glass tube yielded an approximate pumping volume of 148 μl/pump which (with the above periodicity) gives a rate of 21 ml/hr.

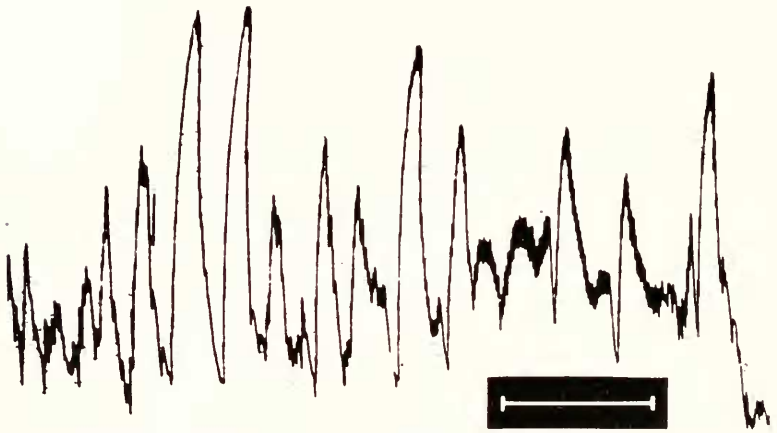

FIGURE 1. Kymographic recording of *Haloclava producta* irrigating an artificial burrow. Scale indicates one hour.

Kymographic recordings of irrigation behavior (Fig. 1) show that irrigation can continue for substantial periods uninterrupted. Indeed, our longest recordings of continued irrigation activity exceed 24 hrs. Quantification of irrigation rate from calibrated kymograph records gives a pump volme of 127 ± 11.8 μl (based on 42 pumps) and a frequency of 71.2 ± 5.0 pumps/hr (based on 5 hr recording time) for an irrigation rate of 9.0 ml/hr. Since a typical 0.5 g animal consumes 15 μl O_2/hr, it must utilize approximately 33% of the 50.5 μl O_2 made available by irrigation of the burrow with air saturated water from above.

Peristaltic waves are not uncommon in anthozoans, having been reported in a pennautlid, *Pteroides griseum* (Brafield and Chapman, 1967), and waves from pedal disc to oral disc are known to aid in body wall cleansing and defecation in *Metridium senile* (Batham and Pantin, 1950b). We are not, however, familiar with any previous report implicating peristaltic waves in burrow irrigation by anthozoans.

Distribution of oxygen

The oxygen content of interstitial water in Waquoit Bay is low. At 5 cm depth in the sand the interstitial pO_2 ranges from 0–5.1 mm Hg, and at 10 cm depth it ranges from 7.5–12.0 mm Hg. These values compare well with those of Brafield (1964), whose study of interstitial oxygen levels in the Scilly Isles included sites of virtually identical sediment composition. Comparable values in similar sediments have also been recorded elsewhere (Eliassen, 1956; Mangum, 1964a; Petersen and Johansen, 1967). Water overlying the sand at the sampling site is over 90% air saturated. Similarly, water in the running seawater system at the Marine Biological Laboratory is virtually air saturated (Fig. 2).

The oxygen content of water in artificial burrows occupied by *Haloclava producta* is equivalent to 128 ± 8 (S.E.) mm Hg. This value does not include an incomprehensible set of measurements on one animal that did not measurably irrigate and in whose tube the pO_2 varied between 46 and 60 mm Hg. The

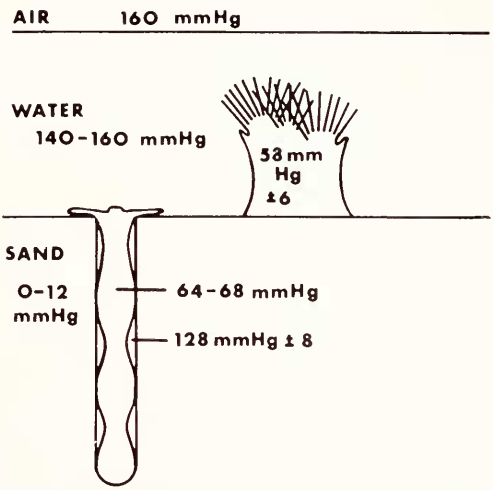

FIGURE 2. Distribution of oxygen in water, sand, burrow and animals. S. E. given for all sample sizes greater than 5, otherwise range.

relatively high pO_2 of the artificial burrow water suggests that burrow irrigation in *H. producta* is sufficient to maintain a high effective environmental pO_2, at least during periods of tidal submersion. That these high pO_2's are maintained persistently in artificial burrows reflects the moderately low fraction of oxygen removal from the irrigation stream relative to rates of oxygen utilization in other infaunal animals (Mangum, 1964a, 1970). The results from the single anomalous animal suggest that the absence of burrow irrigation is accompanied by a substantial decrease in burrow oxygen concentration.

Internal pO_2's of *H. producta* and *M. senile* equilibrated to running seawater (7.6 ppm) are 64–68 mm Hg and 58 ± 6 mm Hg ($= 3.14$ and 2.75 ppm), respectively. Brafield and Chapman (1965) have shown that *Pennatula rubra* equilibrated to 6.9 ppm has an internal oxygen concentration of 3.1 ppm and that

Pteroides griseum equilibrated to 8.3 and 6.6 ppm has internal oxygen concentrations of 2.15 and 3.95 ppm, respectively. It is interesting to note that in all four anthozoan species for which concentration differences have been estimated, the internal oxygen concentration falls in the narrow range of 41–47% of the external concentration (based on a mean value for *P. griseum*). The various levels of oxygen at all major loci for *M. senile* and *H. producta* are summarized diagrammatically in Figure 2.

Survival

Metridium senile survives low oxygen conditions uniformly for 96 hours. Dosage times above 120 hours produce uniform mortality. *Haloclava producta*

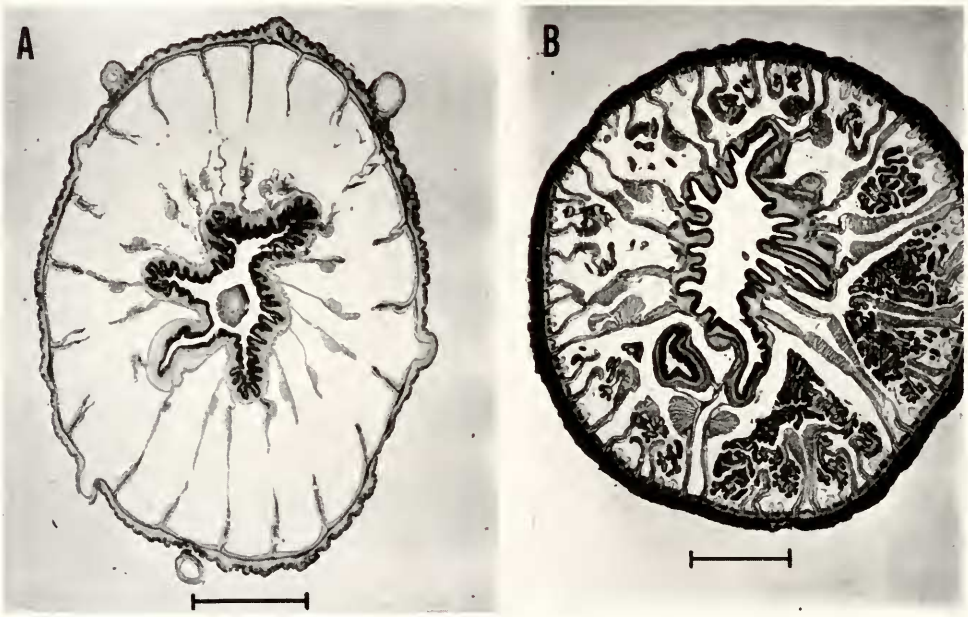

FIGURE 3. (A) Cross section of *Haloclava producta* at the level of the actinopharynx. Scale indicates 1 mm. (B) Cross section of *Metridium senile* at the level of the actinopharynx. Scale indicates 1 mm.

shows no mortality, at least during the first 266 hours; which was the longest dosage attempted. Simultaneous controls show uniform survival.

The resistance of *H. producta* to low oxygen is apparently paralleled by a a resistance to internal hydrogen sulfide. Approximately 120 hours after being introduced into low oxygen water the animals begin to turn black. The coloration begins at the mouth end and slowly proceeds aborally during a period of several days. Dissection of such animals reveals deposits of dark material on the mesenteries and the inside surface of the body wall, in addition to the noticeable odor of hydrogen sulfide. This color change is not accompanied by death and subsequent decomposition. Upon return of intact black *H. producta* to running seawater the

coloration disappears in less than one day, and the animal resumes its normal activities.

During exposures to low oxygen conditions, *H. producta* undergoes marked volume changes. Several days after introduction into such conditions the animals become inflated and distended to a volume about 10 times their body volume in running seawater. There is a threefold increase in body length and a twofold increase in body diameter. Body volume increases occur in *M. senile* under similar conditions, but are less in magnitude. Volume increases in both species are reversed on return to running seawater.

Anatomy

Haloclava producta placed freely in running seawater or anesthesized in $MgSO_4$ is considerably shorter (3–5 cm long) and thicker (0.8–1.0 cm in diameter) than individuals in burrows. There are 20 cylindrical tentacles about 5 mm long and 0.5–1 mm in diameter. Although largely insensitive to touch, the tentacles are retracted under extreme conditions (*e.g.*, chronic exposure to anoxia).

TABLE I

Thermal history and number of animals in each experimental group for measurement of metabolic response

Acclimation temperature (°C)	Experimental temperature (°C)			
	10	17	20	22.5
10	1	4		6
17		8*		
20	3	1	2	5
22.5				2

* Metabolic response determined on same animals after 24 hr exposure to anoxia.

The column is marked by 20 faint lines indicating the insertions of 20 macrocnemal mesenteries. In sectioned material (Fig. 3A) the mesenteries are seen as very thin and delicate septa with small longitudinal retractor muscles. Between the externally visible mesentery insertions are 20 vertical rows of adhesive papillae extending from the oral margin to a variable point down the column, usually one third to one half the total body length. There are 400–600 of these papillae visible on preserved specimens, depending on the particular state of contraction of the specimen. In cross section (Fig. 3A) the papillae appear as hemispherical blisters on the sides of the animal where both the epidermis and the gastrodermis are thinner than elsewhere along the body wall. The body wall thickness of *H. producta* as determined from sections is 141 μ; the thickness at the apex of representative adhesive papillae is 63 μ.

Repeated efforts to detect ciliary currents associated with the tentacles and oral disc of *H. producta* were unsuccessful in both burrowed and non-burrowed animals. Ciliary currents associated with the actinopharynx and mesenterial filaments, however, were easily and repeatedly observed.

Detailed anatomical descriptions of *M. senile* are given elsewhere (Stephenson, 1935; Batham and Pantin, 1951; Hand, 1955b), so only observations pertinent here are discussed below. The general body shape of *M. senile* is considerably less vermiform than that of *H. producta*. The column is smooth with only occasional perforations in the form of cinclides. Tentacles are longer, thinner, and considerably more numerous than those of *H. producta*. They are, in addition, much more readily retracted in response to mechanical stimulation.

The internal organization of *M. senile* (Fig. 3B) is both more complex and more bulky than that of *H. producta*. There are at least 4 cycles of mesenterial septa (96 individual septa) in small individuals. This is five times as many as in *H. producta*. These individual septa are slightly thicker than those in *H. producta* and the cross-sectional area of each longitudinal retractor muscle in the primary and secondary cycles (the 24 largest retractors) is at least 5 times that of the *H. producta* retractors. These numerous mesenterial septa tend to compartmentalize the gastrovascular cavity of *M. senile* to a much greater extent than in *H. producta*. In addition, there is simply a greater bulk of tissue inside *M. senile*. Our sections of *M. senile* are of an individual with a column diameter of 5 mm,

TABLE II

Allocation of surface area

Species	Diameter (mm)	Length (mm)	Number	Surface area (mm^2)	%Total surface area
Haloclava producta					
Body wall	6	100		1884	85.3
Tentacles	1	5	20	314	14.7
Metridium senile					
Body wall	30	40		3768	44.4
Tentacles	0.5	15	200	4710	55.6

the same size as *H. producta*. Hand (1955b) indicates that larger *M. senile* may have several more mesenterial cycles, and since each additional cycle doubles the number of septa, the internal complexity of *M. senile* increases with size as a geometric progression. *H. producta*, on the other hand, has a fixed number of mesenteries (Verrill, 1899).

The easily observable ciliary currents on the tentacles of *M. senile* are directed aborally from the tentacle bases. The oral disc is marked by currents from the mouth running peripherally. The actinopharynx and mesenterial filaments are ciliated and circulation of material along the inside surface of the tentacles was observed in favorable preparations. The body wall thickness is 200–235 μ, almost twice that of non-extended *H. producta*, and an order of magnitude thicker than burrowed *H. producta*.

Several major features of the anatomical organization of the two species are strikingly different. Even the most casual observations under conditions simulating the natural habitat show that anatomical allocation of surface area is distinctly different. Calculations of surface areas of body wall and tentacles for living specimens of each species under natural conditions are shown in Table II. In calcula-

tions for *H. producta,* body wall dimensions are given for the least degree of vermiformicity observed in burrowed animals. For *M. senile,* the body wall dimensions are those of a typical expanded animal and the estimated number of tentacles is intentionally conservative. Stephenson (1935) indicates that the number of tentacles in a crown of *M. senile* ranges up to 700. In both species areas are calculated for epidermal surfaces only. It is clear from Table II that the major contribution to total external surface area in *H. producta* comes from the body wall, and that the tentacle component is rather small. In direct contrast, tentacles of *M. senile* comprise a rather significant component of the total surface area, and in fact their contribution exceeds that of the body wall.

A second major difference in anatomical organization between the two species involves the complexity of their respective internal structure. *M. senile,* which has a greater number of larger mesenteries, is both more compartmentalized and has internalized a greater percentage of its total bulk.

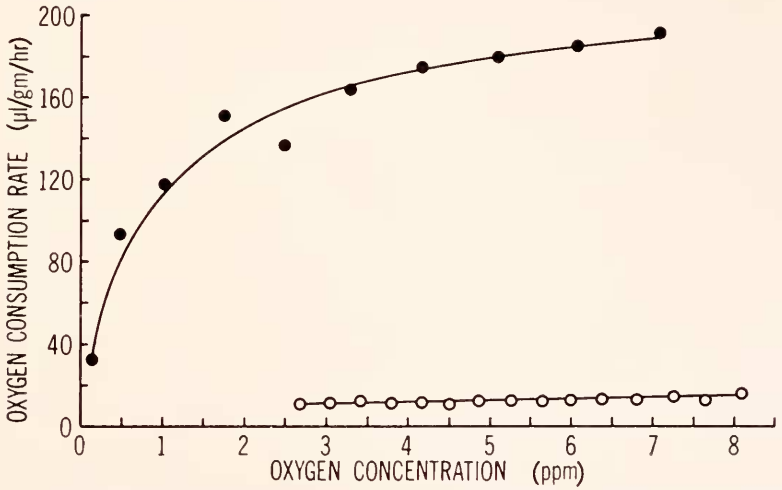

FIGURE 4. The relationship between oxygen consumption rate and oxygen concentration in two individuals of *Metridium senile.* Curves eye-fitted to raw oxygen consumption data.

Oxygen consumption rate and activity at air saturation

The frequency of spontaneous columnar contractions in *Haloclava producta* at 17° C is comparable to analogous measurements at the same temperature in both *Metridium senile* (Batham and Pantin, 1950a) and *Diadumene leucolena* (Sassaman and Mangum, 1970). The oxygen consumption rate of a 1 g *H. producta* at 17° C and air saturation is approximately 30 µl/g/ hr, considerably lower than the rates for *M. senile* (100 µl/g/hr) and *D. leucolena* (50 µl/g/hr) (Sassaman and Mangum, 1970).

Metabolic response to diminishing concentrations of oxygen

Gradual decreases in the oxygen content of ambient water at concentrations near air saturation are reflected in gradual decreases of oxygen consumption rate

in *Metridium senile*. At lower concentrations, the decrease in oxygen consumption rate becomes more pronounced per unit decrease in oxygen concentration (Fig. 4). In this respect *M. senile* resembles the many other organisms which show such partial or imperfect metabolic regulation (C. P. Mangum, 1970; Mangum, Kushins and Sassaman, 1970; Kushins and Mangum, 1971). Oxygen consumption rate is better correlated ($P < 0.001$) with the logarithm of oxygen concentration than the linear axis (Table III). The slope of this semilogarithmic regression (b) is therefore an index of the degree to which oxygen consumption varies with oxygen concentration over the range of air saturation to zero. The coefficient is not a constant, but is highly variable between experiments, ranging from a high of 93.0 to a low of 6.4. When b is high, oxygen consumption rate is greatly affected by changes in oxygen concentration and the metabolic response curve shows partial conformity (Fig. 4, closed circles). When b is low, the oxygen consumption rate is little affected by changes in oxygen concentration (Fig. 4, open circles). The use of b as a quantitative index of sensitivity is valid only if its variation is not a consequence of time dependence in a closed monitoring system,

TABLE III

Fit of linear and semilogarithmic regression lines to relationship of oxygen consumption versus *oxygen concentration according to Student's* t

Species	N	Mean value (± S.E.) of coefficient of determination (r^2)		P level
		semilog	linear	
Metridium senile				
pre-anoxia	32	0.88 ± 0.11	0.75 ± 0.07	<0.001
post-anoxia	8	0.81 ± 0.15	0.69 ± 0.11	>0.10
Haloclava producta				
pre-anoxia	5	0.82 ± 0.19	0.80 ± 0.18	>0.80
post-anoxia	5	0.92 ± 0.21	0.93 ± 0.25	>0.80

or that oxygen consumption is not influenced by the rate of change in oxygen concentration. Figure 5 shows the relationship between b and k. If all 40 experiments are considered as a group, it is readily evident that there is no systematic relationship between oxygen sensitivity (b) and the oxygen depletion constant (k) ($P \geq 0.05$). At any fixed value of k there is no specified corresponding value of b. We regard the data in Figure 5 as strong evidence for the lack of a relationship between b and k and hence as strong support for the use of b as a quantitative measure of oxygen sensitivity. Thus, the observed variation in b is not an artifact, and the question arises as to whether this variation is random or is systematically correlated with environmental factors.

In comparing the various b values from the various experimental groups (Table I) the following relationships were observed: (1) Among groups acclimated to a common temperature, b increases systematically with experimental temperature; (2) Among groups at a constant experimental temperature, b varies inversely with acclimation temperature; (3) Within individual experimental groups, b is negatively correlated with weight ($P < 0.01$); and (4) Between the two groups with

identical thermal histories, high b values are obtained from those animals which were previously exposed to anoxia. In cases (1)–(3), high b values are correlated with conditions known to be associated with high oxygen consumption rates at or near air saturation (Sassaman and Mangum, 1970). In the fourth case, exposure to anoxia also brings about a subsequent elevation in oxygen consumption rate at high oxygen concentration (see below). These various correlations between high b values and elevated oxygen consumption rates at high oxygen concentrations are not coincidental. As shown in Figure 6, b is positively correlated with oxygen consumption rate at 8 ppm ($P < 0.05$). The oxygen consumption rates plotted in Figure 6 are values taken from semilogarithmic regression lines and the value of 8 ppm deviates less than 2.5% from air saturation for all the experimental groups.

Figure 6 clearly shows a linear relation between b (or absolute oxygen sensitivity) and oxygen consumption rate at air saturation (R) such that:

$$b = \frac{R}{1.6} \tag{1}$$

or:

$$\frac{(\Delta\ \mu l/g/hr)}{(\Delta \log ppm)} = \frac{(\mu l/g/hr) \text{ at air sat.}}{1.6} \tag{2}$$

which yields:

$$\frac{(\Delta\ \mu l/g/hr)}{(\mu l/g/hr) \text{ at air sat.}} = \frac{(\Delta \log ppm)}{1.6} \tag{3}$$

Thus, for any fixed reduction of oxygen concentration, Δ log ppm becomes a constant and:

$$\frac{(\Delta\ \mu l/g/hr)}{(\mu l/g/hr) \text{ at air sat.}} = (\text{constant})\ (0.63) \tag{4}$$

Application of equation (4) to the raw data indicates explicitly that, under the conditions employed, *M. senile* responds to a given change in oxygen concentration by changing its oxygen consumption rate by the same percentage which we call c. Thus, although different individuals have different absolute oxygen sensitivities (b) as indicated in Figure 4 and Figure 6, all the animals have the same relative oxygen sensitivity (c). A particular reduction in oxygen concentration elicits the same percentage reduction in oxygen consumption rate in all animals, regardless of thermal history, weight, or previous exposure to anoxia.

Metabolic response to prolonged anoxia

Decreases in the oxygen content of ambient water are reflected in decreases in the oxygen consumption rate of *Haloclava producta*. Perhaps due to the smaller number of observations, we cannot clearly distinguish the best fit (Table III). Semilogarithmic and linear relationships are not significantly different ($P > 0.80$). For comparative purposes, semilogarithmic regression coefficients are used here. Although insufficient data were collected to permit a detailed comparison of the relationship between absolute oxygen sensitivity (b) and oxygen consumption rate

at air saturation (R) in *H. producta versus Metridium senile*, several interesting observations can be made.

In both species, measurable oxygen consumption ceases at oxygen concentrations above zero. Since cessation persists for such long periods, we believe that a switchover to anaerobic respiration occurs in both *M. senile* and *H. producta*, despite the recent contention (Beattie, 1971) that estuarine anemones do not shift to anaerobic pathways during anoxia. The oxygen concentrations at which oxygen consumption ceases are given in Table IV. The residual concentration in *M. senile* is considerably lower than that in *H. producta*, the difference being significant at

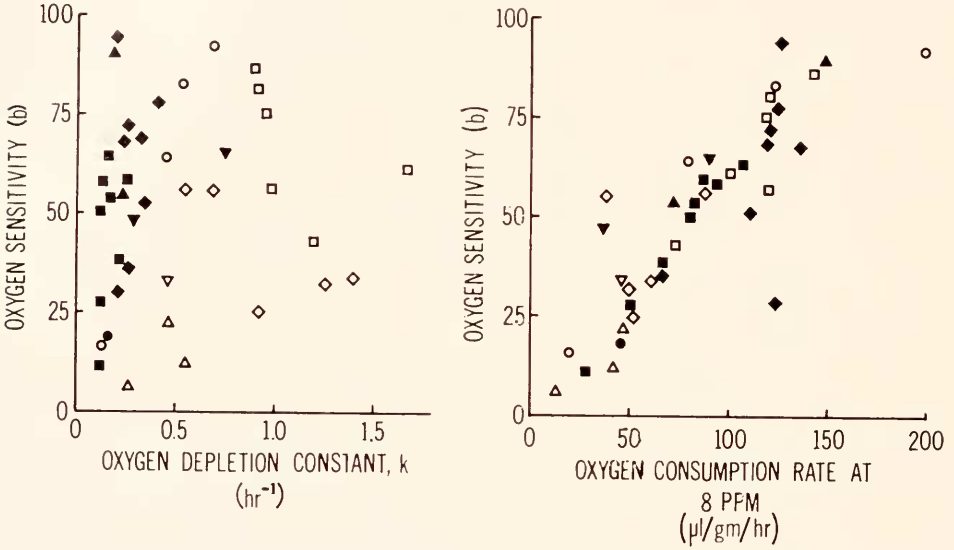

FIGURE 5. The relationship between absolute oxygen sensitivity and the rate constant for oxygen depletion in *Metridium senile*. Closed symbols for animals acclimated to and measured at the following temperatures: circles, 10° C; squares, 17° C; triangles, 20° C; inverted triangles, 22.5° C; diamonds, 17° C (after 24 hr anoxia). Open symbols: circles and squares represent animals acclimated to 10° C and acutely measured at 17° and 22.5° C, respectively; triangles, inverted triangles and diamonds represent animals acclimated to 20° C and subsequently measured acutely at 10°, 17° and 22.5° C, respectively.

FIGURE 6. The relationship between absolute oxygen sensitivity and oxygen consumption rate at 8 ppm in *Metridium senile*. Symbols as described for Figure 5.

$P < 0.001$. In *H. producta* residual concentration is not significantly correlated with body weight, whereas in *M. senile* smaller animals tend to have higher residual concentrations ($P < 0.01$). The difference between the two species may not be real, however, since the number of observations on *H. producta* is smaller. In both species there is a significant ($P < 0.01$) compensatory reduction in residual concentration following anoxia.

Upon re-introduction into air saturated seawater (following a 24 hr period of anoxia), *M. senile* shows the biphasic metabolic response illustrated in Figure 7. The initial phase is marked by a rapid increase in oxygen consumption rate with time, the second phase being characterized by a gradual decrease in oxygen con-

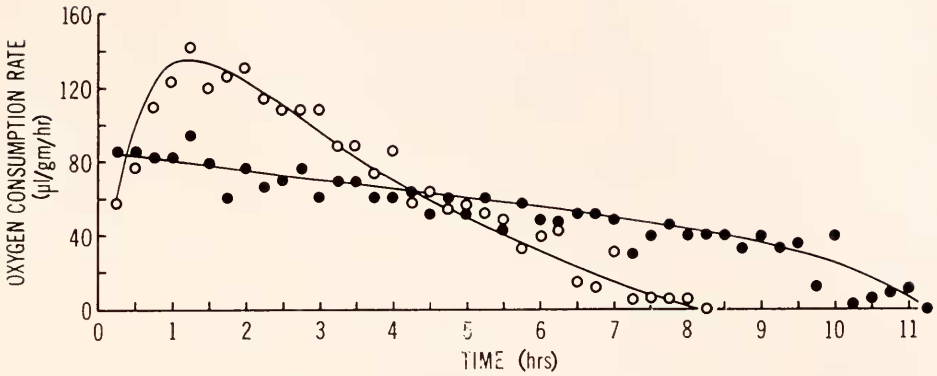

FIGURE 7. Oxygen consumption rate as a function of time in an individual *Metridium senile* before (closed circles) and after (open circles) 24 hr exposure to anoxia (curves eye fitted).

sumption rate as the ambient oxygen concentration decreases. Lag time (the time required to reach maximum oxygen consumption rate) varies in *M. senile* from several minutes to more than one hour and is correlated with animal weight ($P < 0.05$). In only one instance was a lag time recorded in *H. producta*. Since the lag was less than 5 min, it may have been caused by recorder stabilization, but in any event it was far shorter than any lag observed in *M. senile*.

Oxygen consumption rates following anoxia significantly increase in both species. Figure 8 shows the per cent increase in oxygen consumption rate following anoxia as a function of the oxygen concentration. In both species the per cent increase is significantly different from zero at all oxygen levels above 1 ppm ($P < 0.05$) and it varies inversely with oxygen concentration. Both curves in

TABLE IV

Residual oxygen concentrations before and after exposure to low oxygen conditions

Species	Wet weight (g)	Residual concentration before exposure (ppm)	Residual concentration after exposure (ppm)	Change in residual concentration (ppm)
Metridium senile				
	1.86	0.79	0.52	−0.27
	1.94	0.21	0.00	−0.21
	1.95	0.78	0.00	−0.78
	2.68	0.34	0.17	−0.17
	3.26	0.30	0.18	−0.12
	3.38	0.24	0.16	−0.08
	4.09	0.79	0.04	−0.75
	5.00	0.17	0.00	−0.17
	5.66	0.25	0.00	−0.25
Haloclava producta				
	0.392	2.14	0.37	−1.77
	0.474	0.98	0.08	−0.90
	0.521	1.80	0.20	−1.60
	0.611	1.59	0.63	−0.96
	0.635	0.73	0.05	−0.68

Figure 8 asymptote at oxygen concentrations where oxygen consumption rates become very low (Table IV). It is only at these asymptotes that the percent increase in oxygen consumption becomes less than significant ($P > 0.05$). The absolute increase in oxygen consumption rate following anoxia is shown as a function of oxygen concentration for both species in Figure 9. The absolute increase is significantly different from zero at all oxygen concentrations in both species indicating that the lack of significance at 1 ppm in Figure 8 is a statistical artifact. Interestingly enough, the variation in absolute increase between individuals of the same species is surprisingly low.

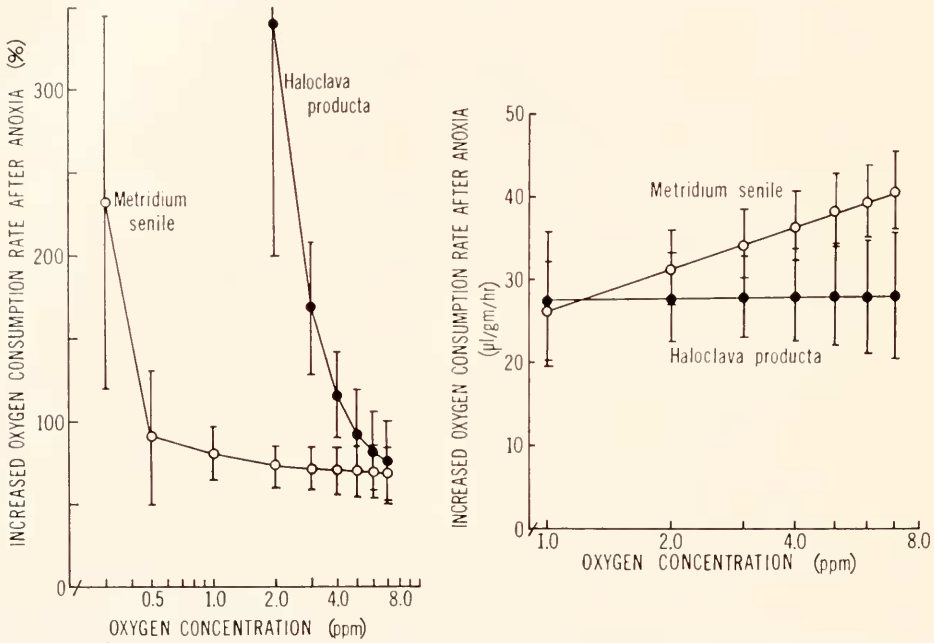

FIGURE 8. The relative increase in oxygen consumption rate after 24 hr anoxia as a function of oxygen concentration (vertical bars ± S.E.).

FIGURE 9. The absolute increase in oxygen consumption rate after 24 hr anoxia as a function of oxygen concentration (vertical bars ± S.E.).

The changes in absolute oxygen sensitivity (b) of both species after exposure to 24 hr anoxia are given in Table V. In *M. senile* there is a significant increase in b ($P < 0.05$) in 4 of 8 anemones. In 7 of the 8 individuals there is an increase in b, and in the one instance where a decrease is noted, the change is non-significant ($P > 0.05$). It seems that the general response of *M. senile* to prolonged exposure to anoxia includes a subsequent increase in absolute oxygen sensitivity (b), although the response is not entirely invariant. However, as we have already shown (Fig. 6 and Equation 4) the relative sensitivity to reduced oxygen concentration (c) does not change after exposure to anoxia.

In *H. producta*, on the other hand, the changes in oxygen sensitivity following anoxia are much more variable. Absolute oxygen sensitivity (b) increases sig-

nificantly ($P < 0.05$) in one instance, decreases significantly ($P < 0.05$) in two instances, and remains relatively unchanged in the two remaining instances (Table V). This inconsistency of response in *H. producta* is very similar to that in *Nassarius obsoletus* and *N. trivittatus*, two snails which often burrow into low oxygen mud (Kushins and Mangum, 1971. The inconsistent response of *H. producta* contrasts markedly with the more stereotyped increase in absolute oxygen sensitivity in *M. senile*. Furthermore, since oxygen consumption rate consistently increases after anoxia in *H. producta* (Fig. 9) but absolute oxygen sensitivity (b) does not, it is clear that there is no single relative oxygen sensitivity (c) for *H. producta*.

TABLE V

Change in absolute oxygen sensitivity following anoxia

Species	Oxygen sensitivity before anoxia (b)	Oxygen sensitivity after anoxia (b)	Change (b)	Statistical significance (P)
Metridium senile				
	53.74	72.48	18.74	<0.05
	57.61	94.02	36.41	<0.05
	11.44	35.97	24.53	<0.05
	27.13	77.22	50.09	<0.05
	57.99	69.50	11.51	ns
	63.76	68.10	4.34	ns
	37.74	51.75	14.01	ns
	50.57	29.19	−21.38	ns
Haloclava producta				
	38.07	97.44	59.31	<0.05
	42.78	46.37	3.59	ns
	111.55	76.55	−35.00	<0.05
	38.55	22.78	−15.77	<0.05
	85.33	82.55	−2.78	ns

DISCUSSION

Metridium senile is continuously bathed in water of relatively high oxygen content (140–160 mm Hg) except during brief periods of intertidal exposure. Although Parker (1922) has shown measurable CO_2 production in *M. senile* under aerial conditions, the relationship between submerged and aerial aerobic metabolism in this species is not known. In any event, the prevailing oxygen content of the *M. senile* environment is constantly at or near air saturation.

It is clear from Figure 2 that irrigation by *H. producta* is sufficient to maintain quite high pO_2's in artificial burrows. In natural burrows it is doubtful that loss of burrow oxygen to surrounding low oxygen sediments is sufficient to lower appreciably the oxygen levels of the burrow while the animal is irrigating. The occupation and irrigation of a burrow, however, would tend to require certain behavioral and morphological adaptations which might be evident in a comparison between *M. senile* and *H. producta*. In addition, the oxygen content of the *H. producta* environment might well be less constant and less predictable than that of *M. senile*. Conditions which interrupt irrigation behavior lead quite rapidly

to low burrow pO_2's. Although our kymographic recording suggest long periods of uninterrupted irrigation in a shaded area of the laboratory, irrigation may not be continuous in the field. Hargitt (1907) has described the importance of negative photo-tropisms in *H. producta* behavioral patterns. In addition, periods of intertidal exposure and storm shifted sediments would force cessation of burrow irrigation. Given these considerations we might expect to find two types of adaptations in *H. producta* relative to *M. senile:* (1) those facilitating extraction of oxygen from an irrigation current, and (2) those allowing for a rapid "rebound" from fortuitous periods of anoxia.

The major contribution to surface area in *H. producta* comes from the body wall whereas in *M. senile* the tentacles are of greater importance. The dichotomy becomes even more striking when we note that the body wall of *H. producta* in its burrow is at least one order of magnitude thinner than that of *M. senile*, that the body wall of *H. producta* is studded with several hundred even thinner walled papillae, and that *H. producta* shows no apparent tentacular ciliation whereas *M. senile* does. These morphological features suggest a major difference in the site of oxygen diffusion into the two species: exchange in *M. senile* is typically localized to the tentacles but it is spread out more linearly in the elongate *H. producta.*

Both *M. senile* and *H. producta* show volume increases on exposure to low oxygen conditions, but the response is much more stereotyped and extreme in *H. producta.* Such marked volume increases have two major effects on the respiratory properties of *H. producta.* First, the surface area to weight ratio is greatly increased, allowing a greater area for diffusion. Secondly, the diffusion distance across the body wall is reduced, thereby increasing body wall permeability. Both of these effects greatly increase the oxygen exchange capacity of *H. producta* upon resumption of burrow irrigation, as suggested by the rarity or absence of a lag time.

A second major anatomical difference between *M. senile* and *H. producta* involves the complexity of their respective internal organizations. As in most anthozoans, the enteron is open and continuous, and only partially compartmentalized by the mesenterial septa. As Stephenson (1921) has pointed out, ciliary activity of the mesenterial filaments probably helps maintain the continuity of the enteric fluid by keeping apart adjacent septa. Nevertheless, the degree to which the enteron is compartmentalized may be related to mesenterial organization. Internal mixing of the open enteron and access of enteric water to spaces between mesenteries may be facilitated by the lack of complex organization in forms such as *H. producta.*

The observation that contracted pennatulids, in which enteric fluid circulation is greatly decreased, have low metabolic rates (Brafield and Chapman, 1965) suggests that the major site of oxygen consumption is gastrodermal. In actinians the bulk of tissue is gastrodermal; specifically, half of the body wall and tentacles, and all of the mesenteries are gastrodermal (Stephenson, 1928), if mesoglea is discounted. We have no data from this or previous studies (Sassaman and Mangum, 1970) to suggest that appreciable amounts of oxygen are exchanged gastrodermally in actinians. In our continuous recordings of oxygen consumption there is no indication of any periodic rapid decrease in oxygen concentration as has been demonstrated in pennatulids (Brafield and Chapman, 1967) and renillids (Chapman, 1972). Furthermore, it is unlikely that continuous peristaltic irriga-

tion waves, such as we record from *H. producta*, are generated by an animal continuously varying its body volume (and hence hydrostatic pressure) by ventilation of the gastrovascular cavity. In addition, the frequency of large scale emptying of the gastrovascular cavity of *M. senile* is low (Batham and Pantin, 1950b) and it is unlikely that the volume of water exchanged during minor columnar contraction carries an appreciable amount of the total requisite oxygen into the gastrovascular cavity for direct transfer to the gastrodermis.

Actinians seem to be faced with the problem of transporting and delivering oxygen, which is procured primarily at an epidermal site, to a primarily gastrodermal site of consumption. Morphological adaptations in a burrowing anemone might therefore tend to maximize the epidermis/gastrodermis ratio and maximize gastrovascular continuity. In addition, one might expect enhancement of gas exchange via thinning of the body wall and a shift in the site of oxygen exchange to the body wall as adaptations for rapid re-oxygenation of gastrovascular fluid in a burrow-irrigating anemone.

Vermiformicity and simplicity of internal organization are certainly not obligatory features of burrowing anemones. Indeed, there are structurally rather complex anemones which exhibit the burrowing habit (Hand, 1955a; D. C. Mangum, 1970), and in some other anthozoans direct ventilation of the gastrodermis seems a viable alternative (Parker, 1920; Brafield and Chapman, 1967). In fact, within the sea anemones there is a phylogenetic component associated with many of these morphological correlates of burrowing. Halcampids and haloclavids, both of which show vermiformicity and simplicity of internal organization, are more closely related to each other phylogenetically than either is to some of the more structurally complex burrowing anemones (Stephenson, 1921). Nevertheless, the kinds of structural features emphasized here cut across phylogenetic lines. Vermiformicity and internal simplicity are characteristic of edwardsids, an anthozoan line distinct from that of the halcampids and haloclavids (Stephenson, 1921). Parallel construction is found in the glycerid polychaetes in which typical annelid vermiformicity is accompanied by metameric distribution of the gills and loss of intersegmental setpa. A very striking parallelism occurs in such synaptid holothuroids as *Leptosynapta*, in which the perivisceral coelom is almost totally uncompartmentalized via loss of the bulk of the hemal and water vascular systems, the respiratory trees are lost, the surface area of the tentacles greatly reduced and the body elongated. Similar vermiformicity and simplicity of internal organization is characteristic of the meiofauna inhabiting the interstitia of marine sediments (Swedmark, 1964). The modifications which we see in *H. producta* are apparently representative of a general pattern or strategy of adaptation to infaunal existence and they are not a specific solution employed by one group of sea anemones.

Comparison of physiological properties of *H. producta* and *M. senile* support the same general conclusion which have emerged from other studies comparing species from stable high oxygen environments with closely related forms living in lower or less predictable oxygen environments. Although both species survive anoxia for periods considerably in excess of those generated by tidal cycles, survival of *H. producta* is at least twice that of *M. senile*. In addition, at comparable levels of columnar activity and oxygen saturation, *H. producta* has a lower oxygen consumption rate than *M. senile* of the same body size. There is no apparent corre-

lation between environmental oxygen level and absolute oxygen sensitivity. The variation in absolute oxygen sensitivity (*b*) confirms the findings of numerous studies which have shown that there is no constant value in a species for the relationship between oxygen consumption rate and oxygen concentration (Helff, 1928; Hiestand, 1931; Hyman, 1932; Lindeman, 1935; Thomas, 1954; Van Dam, 1954; Wiens and Armitage, 1961; Eriksen, 1963; Nimura and Inoue, 1969; Thompson and Pritchard, 1969; Bayne, 1971). Of particular interest, however, is the finding of a single relative oxygen sensitivity (*c*) in *M. senile* which is independent of acclimation temperature, experimental temperature, weight and previous exposure to anoxia. A comparable constant is not found in *H. producta*. Its absence may characterize species inhabiting low or unpredictable oxygen environments, but interpretation of the ecological correlation is not yet obvious.

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Summary

1. Aerobic metabolism in *Haloclava producta*, a burrowing sea anemone, is largely or wholly dependent upon the oxygen supply in the water overlying its burrow.

2. This superficial water is brought through the burrow by an irrigation cycle of peristaltic waves. About 33% of the oxygen in the irrigation current is withdrawn by the animal.

3. Externally *H. producta* is exceedingly vermiform, studded with numerous thin walled hollow blisters and body surface area largely consists of the columnar body wall. *M. senile*, in comparison, is more robust, relatively smooth walled, and most of its total surface area is tentacular. Internally, the body wall of *H. producta* is an order of magnitude thinner than that of *M. senile*, and *H. producta* has fewer and smaller mesenterial septa and longitudinal retractors.

4. These morphological considerations suggest that vermiformicity and simplicity of internal organization are adaptive to low or unstable oxygen environments. The modifications associated with *H. producta* are not uniquely actinian, and are paralleled in completely unrelated phyla; thus they may represent a general mode or pattern of adaptation to burrow existence.

5. The morphological adaptations seem to be correlated with efficient use of the oxygen in the irrigation current, and with rapid restoration of internal pO_2's following transient periods of anoxia.

6. *H. producta* is much more resistant to prolonged anoxia than *M. senile*, and it has a lower oxygen consumption rate at air saturation.

7. After exposure to anoxia, both species show a compensatory increase in the fraction of dissolved oxygen which they can remove from a closed system.

8. After exposure to anoxia, both species show an increased rate of oxygen consumption (both in relative and absolute terms) over wide ranges of oxygen concentration.

9. Different individuals of *M. senile* have very different absolute metabolic response curves to diminishing oxygen concentration, but all individuals examined showed the same relative decrease in oxygen consumption rate over a given change in oxygen concentration. This relative change is independent of acclimation temperature, experimental temperature, weight and previous exposure to anoxia. Different individuals of *H. producta* also have different absolute metabolic response curves to diminishing oxygen concentration, but a single relative response comparable to that in *M. senile* does not exist.

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